

Physical Characteristic

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Physical Characteristics of Phycocyanin from *Spirulina* Microcapsules using Different Coating Materials with Freeze Drying Method

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Abstract : The aim of this study was to compare the physical characteristics of phycocyanin microcapsules (F) from *Spirulina* sp. with different coating materials, such as κ-Carrageenan (C) and Na-alginate (A) in combination with maltodextrin (M) by freeze drying method. Microcapsules were prepared in three variations of coating materials i.e. maltodextrin (FM); maltodextrin and Na-alginate (FMA); and maltodextrin and carrageenan (FMC) with concentration of each materials were 10%; 9%:1.0%; and 9%:1% (w/w), respectively. The results showed that FMA with Na-alginate 1.0% produced the highest bulk density and total soluble solid, there were 0,334 g/ml and 9,067%, respectively. Color analysis by chromameter showed that FMC produced the bluest color compared to other samples. The glass transition temperature (Tg) investigated with Differential scanning calorimeter (DSC) in all of the samples.

Keywords: Carrageenan, Maltodextrin, Na-alginate, Physical Characteristics, Phycocyanin

1. Introduction

Food coloring materials both naturally or synthetic frequently used in daily life. The color is an important part which affects the product appearance, either for food, beverages, cosmetics, or drugs. Synthetic dyes often used because the price is lower than natural dyes, provides pleasing color, and more stable. However, many synthetics caused an allergic reaction when exposed to the human, even in particular cause also can be carcinogenic.

Blue synthetic dyes do not many in number, including *Brilliant Blue* and *Indigotine*. *Brilliant Blue* resistance in light and acidity, while *Indigotine* does not resist in light, heat and acidity. Both of these synthetic blue dyes do not resist to oxidation [1], so by consuming synthetic dye in high amount can harmful to health. Natural dyes can be alternative because it's safer and has beneficial effects on health.

Phycocyanin is natural blue pigment contained in *Spirulina* sp., which able to be as antioxidant [2]. Phycocyanin not stable against pH, temperature, light and humidity [3,4]. Phycocyanin has potential as a natural dye, but the effort required to maintain phycocyanin during storage.

Microencapsulation is one of an alternative to process phycocyanin into powder, which has a particle size between 1 – 1000 μm [5]. Microencapsulation process requires coating materials to forming microcapsules. The coating materials used can influence the physical properties of microcapsules. Maltodextrin is coating materials often used in microencapsulation process due to its ability to forming a film and easily soluble in water. Carrageenan able to forming a gel, coagulate and often used as stabilizing agent [6]. Alginate has biocompatibility properties and easily to forming a gel [7].



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Maltodextrin will be combined using other coating materials like carrageenan and natrium alginate in order to improve its ability to protect phycocyanin released out.

Some studies using spray drying method to process microencapsulation [8,9,10,11] because of its affordable price and the availability of equipment. However, spray drying using high inlet temperature, which can reduce the phycocyanin content. One of an alternative method that can utilize is freeze drying. This process consists of freezing and sublimation, which is known as an efficient method to protect enzyme [12] and antioxidant [13]. Freeze drying method also is known to maintain the anthocyanin content rather than spray drying method [14]. The aim of this study was to compare the effect of different coating material used using freeze drying method into the physical properties of phycocyanin microcapsules.

2. Methods

2.1. Phycocyanin Microencapsulation

10% (w/v) of maltodextrin DE 10 (CV. Multi Kimia Raya, Semarang, Indonesia) mixed with phycocyanin extracted from *Spirulina* sp. powder (PT. Neoalga, Sukoharjo, Indonesia). κ -Carrageenan and Na-alginate (PT. Selalu Lancar Maju Karya, Jakarta, Indonesia) each combined with maltodextrin as coating materials then was coded as FMC (Phycocyanin microcapsules using maltodextrin and κ -Carrageenan with concentration 9%:1%) dan FMA (Phycocyanin microcapsules using maltodextrin and Na-alginate with concentration 9%:1%). The emulsion was then microencapsulated using freeze dryer (Ningbo Yinzhou Sjia Lab Equipment Co., LTD).

2.2. Yield

Yield was measured by Nunes and Mercadante [15]. The yield was calculated based on the percentage ratio of microcapsules phycocyanin mass with total solids solution of phycocyanin microparticles.

2.3. Bulk Density

Bulk density was measured by using Rao and Vidhyadhara [16] method with modification in the tube used. Phycocyanin microcapsules placed on the 10 ml tube then measured its weight. Bulk density calculated by dividing the microcapsule weight and volume and its expressed in gram per cm³.

2.4. Color with chromameter

Phycocyanin microcapsules color was determined by chromameter CR-200 (Minolta), which measuring of L, a, and b level. The L level indicates the brightness level, a negative level indicates towards green color, b negative level indicates blue color, and b positive indicate yellow color.

2.5. Dissolved Solids

0,5 g samples and 6 mL distilled water put into 100 mL of a flask, then shaken and heated in a water bath for 5 minutes. Once cold, distilled water added up to the limit line then filtered. Next, 5 ml of substrate was taken and poured into porcelain dish which is known its weight (A) g. The filtrate evaporated in the water bath until dry. Then dried in the oven at 105° until its weight constant (B) g. The dissolved solid level calculated as follow [17]:

$$DS (\% \text{ dB}) = (B-A)/\text{dry weight sample} \times 10/0,5 \times 100\%$$

2.6. Encapsulation Efficiency (EE)

The Encapsulation Efficiency was measured by Yan *et al.*, [4]. The EE is calculated based on the presentage ratio of non coated phycocyanin mass and phycocyanin mass added at the beginning of the microencapsulation process.

2.7. Differential Scanning Calorimeter (DSC)

Maltodextrin, carrageenan, and phycocyanin microcapsule were thermal analyzed using DSC. The thermogram of each sample measured at a temperature range of 30°C to 300°C with the speed of 10°C per minute [18].

2.8. Statistical Analysis

Those data were analyzed using SPSS 17 with triplication. Data were analyzed using one-way ANOVA with Tukey test.

3. Results and Discussion

Table 1. Bulk Density and DS Analysis Result

No	Sample	Yield (%)	Bulk Density (g/ml)	DS(%)	Encapsulation efficiency/EF (%)
1	FM	84,723 ± 5,738 ^a	0,193 ± 0,110 ^c	7,867 ± 0,115 ^b	30,787 ± 0,311 ^a
2	FMA	83,093 ± 2,296 ^a	0,334 ± 0,006 ^a	9,067 ± 0,306 ^a	35,917 ± 0,391 ^b
3	FMC	79,787 ± 2,678 ^a	0,306 ± 0,003 ^b	7,200 ± 0,200 ^c	39,070 ± 3,725 ^c

Note: The data was the average of triplication ± standard deviation.

Different superscript on the same column indicates significantly different at level of α 0,05

3.1. Yield

Based on phycocyanin microencapsulation yield calculation, its showed there was no significant different result between FM, FMA, and FMC treatments. Those result indicated that different coating materials used in the same concentration in freeze drying method didn't influence phycocyanin microcapsule yield (Table 1). Phycocyanin microencapsulation using freeze drying method and different coating materials could produce more than 79% of microcapsule yield. On the otherhand, the yield obtained from microencapsulation of some materials using freeze drying method was not more than 50% [19,20]. The yield finding in this research was lower than others researcher since the inlet temperature used is not stable, hence causing the product sticky on the spray dryer chamber. Some microcapsule product are also lost because it can't separate on the cyclone, so the microcapsule flies into the air and enters the filter tube. Therefore, it is leading to losses the product during the process [20,14]. In this case, it was not found on freeze drying microencapsulation because there was no drying materials flow and product that produce as spray drying methods.

3.2. Bulk Density

Table 1 showed phycocyanin microcapsule bulk density level. Among the results, FMA had high bulk density, followed by FMC and FM respectively. Alginate and Carrageenan are polysaccharides which able to form a gel, where its functional properties influenced by its unique structure [22]. These properties able to trapped phycocyanin and avoid the moisture loss during freeze drying process. The bulk density was also influenced by the water holding capacity (WHC) level from emulsion which is formed by alginate and carrageenan. Amid *et al.*, [23] explained that sodium alginate WHC level is higher than κ -carrageenan which affects the microcapsule weight particle thereby increasing the bulk density value.

3.3. Dissolved Solids (DS)

Ease of phycocyanin microcapsule water soluble indicated by dissolved solids (Table 1). The highest to the lowest dissolved solids were FMA, FM, and FMC, respectively. Alginate can dissolve in hot water [24], so the combination between maltodextrin and alginate can produce high dissolved solids. Water

solubility related to bioactive components release speed [25], so the larger dissolved solids, the faster phycocyanin released.

Bioactive release rate, also influenced by used coating materials concentration. According to Mandal *et al.* [26] the lower alginate concentration used, the faster drugs release, where 2% of alginate concentration produce the fastest drug release. Carrageenan has a water soluble properties and able to forming a gel [27] which caused FMC had the lowest dissolved solids. The low dissolved solids of FMC caused slow phycocyanin to release.

3.4. Encapsulation Efficiency (EE)

Encapsulation efficiency showed that the amount of phycocyanin that could be encapsulated, which the high of EE, the phycocyanin that could be encapsulated rise excessively (Table 1). This result indicated that in the same concentration, κ -carrageenan has higher ability to encapsulate phycocyanin using freeze drying method compared to those α -alginate and plain maltodextrin. EE influenced by the type of polymer that using as the coating materials which could affect the hydrophobic characteristic of emulsifier used. Some of the polymer types had an emulsifying ability and maintaining the viscosity of the emulsion. EE is also likely to be affected by physic-chemical factors governing the encapsulation between the biopolymers used as coating material and active compounds [28,29,30].

Phycocyanin microcapsules, there were FM, FMA, and FMC had EE value 30,787%, 35,917%, and 39,070%, respectively. This result was lower than Holkem *et al.*, [31], where the EE of alginate microcapsules with freeze drying method was 89,71%. According to Karthik and Anandharamakrishnan [32], an active compound which encapsulated using freeze drying method could produce porous microcapsule on its surface because there is ice sublimation during the drying process. Which lead the active compound oxidation. Otherwise, needs coating material which protects phycocyanin during freeze drying method.

3.5. Color

Phycocyanin microcapsule color was presented Table 2. It can be seen that FMC phycocyanin microcapsule had the bluest color compared to FM and FMA. Those finding showed that carrageenan able to protect phycocyanin during freeze drying process due to carrageenan ability to forming a gel.

Table 2. Phycocyanin Microcapsule Color Level

No	Sample	L	a	b
1	FM	66,313 \pm 0,185 ^a	-4,397 \pm 0,349 ^a	-12,520 \pm 0,486 ^b
2	FMA	71,160 \pm 0,527 ^b	-6,397 \pm 0,067 ^c	-9,500 \pm 0,060 ^a
3	FMC	72,943 \pm 0,061 ^c	-5,133 \pm 0,025 ^b	-13,527 \pm 0,127 ^c

Note: The data was the average of triplication \pm standard deviation.

Different superscript on the same column indicates significantly different at level of $\alpha = 0,05$

Regarding different characteristic, some polysaccharides are able to form a gel in different type [27]. The κ -carrageenan was able to forming stable gel because of 3,6 *anhydrogalactose* and *galactose 4 sulfate* content [33]. According to Mehrad *et al.*, [25] the combination between maltodextrin and κ -carrageenan produce fish oil microcapsule with the most yellow color than the mix between maltodextrin-gelatin and maltodextrin-gelatin- κ carrageenan.

5 Unlike the κ -carrageenan, alginate is polysaccharides extracted from brown seaweed, which contains β -D-mannuronic acid (M) and α -L-guluronic acid (G). The comparison between M / G content of alginate will affect the gel properties result. The lower ratio of M / G of alginate will produce a weak gel. Furthermore, Fertah *et al.*, [34] reported that alginate with M / G 1,12 will bring a soft and elastic gel. In this study, FMA had the lowest blue color, so the combination between maltodextrin and alginate were less able to protect the phycocyanin because of weak gel strength.

3.6. Differential Scanning Calorimeter (DSC)

Phycocyanin microcapsule DSC Thermogram with different coating materials showed in Figure 1. FM microcapsule produces two onsets temperature of 140,93°C and 152,32°C which showed there were two components in the microcapsule of phycocyanin and maltodextrin. According to Kouassi *et al.*, [35] onset temperature showed glass transition temperature (T_g). Two onset temperature formation of DSC Thermogram showed there were two components on microcapsule. The FMA and FMC microcapsule had one onset temperature of 141,77°C and 137,04°C, respectively. One onset temperature formation revealed that phycocyanin was entirely dispersed in used coating materials. Devi and Kakati, [36] stated that there was no ascorbic acid onset temperature formation of ascorbic acid microparticles showed that ascorbic acid dispersed was into microparticles.

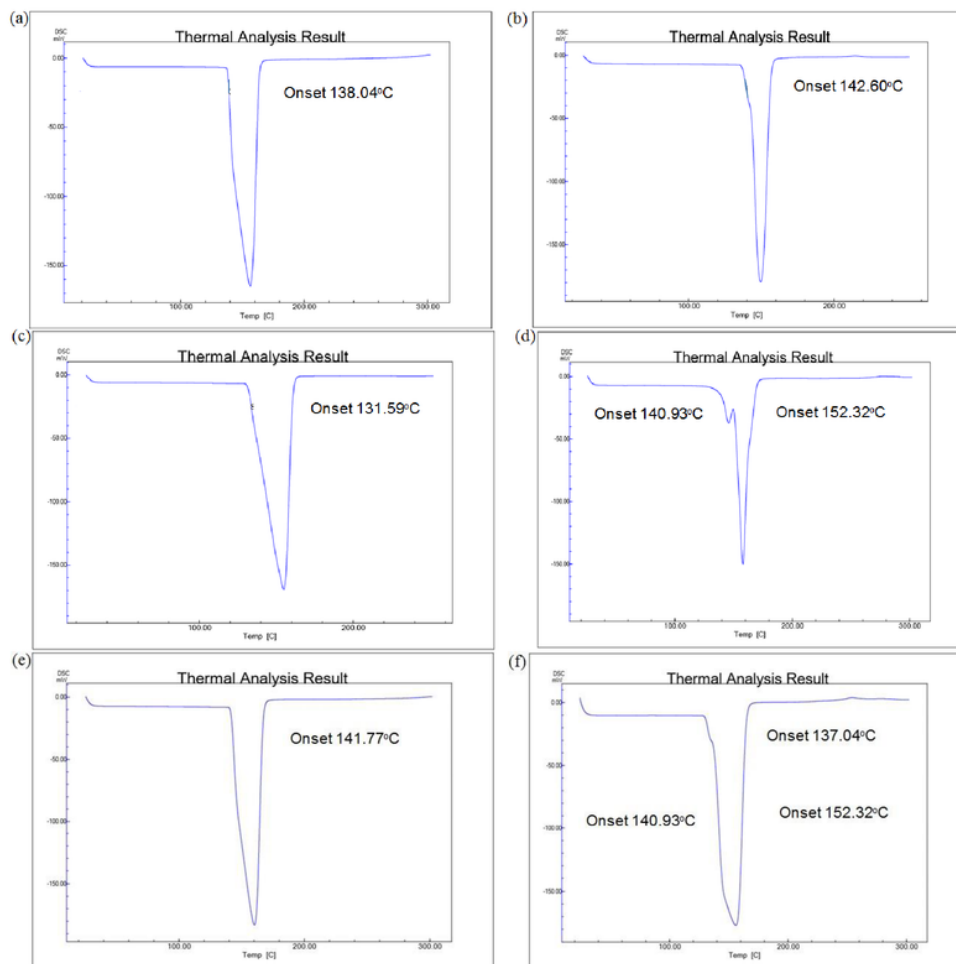


Figure 1. DSC Thermogram (a) alginate (b) κ -carrageenan (c) maltodextrin (d) FM (e) FMA (f) FMC

4. Conclusion

Phycocyanin microcapsules using maltodextrin and alginate coating materials produce the highest bulk density and dissolved solids. Phycocyanin microcapsules using maltodextrin and κ -carrageenan coating materials produce the bluest color. Based on DSC analysis, the combination between maltodextrin-alginate and maltodextrin- κ -carrageenan produce one onset temperature which showed that phycocyanin was perfectly dispersed in coating materials used. Based on that result, alginate and κ -carrageenan were potential as coating materials for phycocyanin microcapsule process.

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